IN THE U.S. PATENT & TRADEMARK OFFICE

Applicants:	Yukoh HIEI et al		
Serial No.:	10/089,696	Group:	1661
Filed:	July 24, 2002	Examiner:	Kubelik

For: Method for Promoting Efficiency of Gene Introduction into Plant Cells

DECLARATION UNDER 37 C.F.R. § 1.132

Honorable Commissioner of Patents and Trademarks

Washington, D.C., 20231

Sir:

I, Yukoh HIEI, a nation of Japan, residing at c/o Japan Tobacco Inc., Plant Breeding and Genetics Research Laboratory, 700, Higashibara, Toyoda-cho, Iwata-gun, Shizuoka 438, Japan, do hereby declare as follows:

I am a co-applicant of the invention as described and claimed in the specification of the above-identified application.

I am familiar with the Final Office Action dated February 22, 2008, in which claims 22 - 30 are rejected.

To show the patentability of the present invention, I carried out the experiments described below.

Materials and Methods

(1) Sample Varieties and Tissue

As the sample varieties, Koshihikari and IR64, which are the varieties of Japonica and Indica rice respectively, were used. As the sample tissue, immature embryo was used. The preparation method of the tissue is the same as that described in the specification of the present patent application.

(2) Centrifugation Treatment and Culturing

Rice immature embryos were placed in a 1.5 ml centrifugal tube containing 1 ml of sterilized water. For variety Koshihikari batches of 10 immature embryos were centrifuged at 20,000 xg for 10 min and then cultured on the callus induction medium N6-As (N6 salts and vitamins, 0.5 g l^{-1} vitamin assay casamino acids, 0.5 g l^{-1} L-proline, 20 g l^{-1}sucrose, 10 g l^{-1} D-glucose, 1 mg l^{-1} 2,4-D, 0.5 mg l^{-1} NAA, 0.1 mg l^{-1} BA, 0.1 mM acetosyringone, 8.0 g l^{-1} agarose Type I, pH5.2.), which is available for use in co-cultivation, at 25 °C in the dark for a week. Non-centrifuged control batches of embryos of Koshihikari were cultured in the same way.

Four immature embryos of IR64 were centrifuged at 1,100 xg for 10 min and then cultured on the medium N6-As at 25 °C in the dark for a week. Non-centrifuged control batch of embryos of IR64 were cultured in the same way.

(3) Measurement of Weight and Feature of Cultured Embryos

For variety Koshihikari elongated shoots (hypocotyls) were removed from the embryos after culturing for a week, and the combined weight of 10 immature embryos in a batch was measured. Five batches were measured in each experimental plot. The average weight per batch was compared between the centrifuged and non-centrifuged plots.

For variety IR64 elongated shoots were removed from the embryos after culturing for a week, and a picture of the embryos with the scutellum side up was taken under a stereoscopic microscope.

Results and Discussion

Certain changes were observed in immature rice embryos after centrifugation. Elongation of shoots from the immature rice embryos during the period of co-cultivation usually takes place without pre-treatments in both of Koshihikari and IR64. However, it was not detected after the centrifugal treatment. In addition, callus

formation from scuteller tissue was better on callus induction media from pre-centrifuged embryos than from non-treated embryos again in both both Koshihikari (Figure 1) and IR64 (Figure 2). Clear difference of color on the scuteller tissues between non-treated embryos and centrifuged embryos was also observed (Figure 2).

Thus, it is possible that centrifugation hinders normal differentiation and development of organs but promotes dedifferentiated growth of plant cells, which likely makes plant tissues more competent in transformation. However, nothing is known about the mechanisms involved.

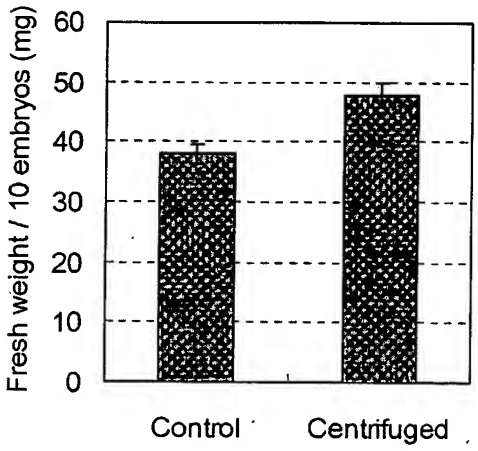

Figure 1. Proliferation of cells from rice immature embryos treated with centrifugation.

Batches of 10 immature embryos of Koshihikari were centrifuged at 20,000 xg for 10min and then cultured on a callus induction medium at 25 °C in the dark for a week. Elongated shoots were removed from the immature embryos, and the combined weight of 10 immature embryos in a batch was measured. Averages of 5 batches and standard errors are plotted.

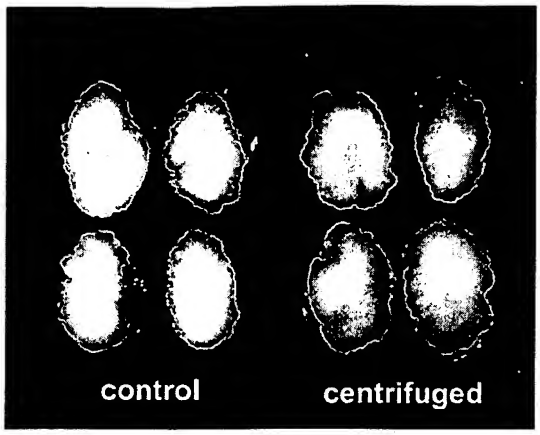

Figure 2. Immature embryos of IR64 after culturing on callus induction medium for a week.

Immature embryos of IR64 were centrifuged at 1,100 xg for 10 min and then cultured on the callus induction medium N6-As at 25 °C in the dark for a week. Non-centrifuged control batch of embryos were cultured in the same way. Elongated shoots were removed from the embryos after culturing for a week, and a picture of the embryos with the scutellum side up was taken under a stereoscopic microscope.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

This 21 day of August, 2008 Yukoh Hiei

Yukoh HIEI